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Role of voltage-gated sodium, potassium and calcium channels in the development of cocaine-associated cardiac arrhythmias

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Cocaine is a highly active stimulant that alters dopamine metabolism in the central nervous system resulting in a feeling of euphoria that with time can lead to addictive behaviours. Cocaine has numerous deleterious effects in humans including seizures, vasoconstriction, ischaemia, increased heart rate and blood pressure, cardiac arrhythmias and sudden death. The cardiotoxic effects of cocaine are indirectly mediated by an increase in sympathomimetic stimulation to the heart and coronary vasculature and by a direct effect on the ion channels responsible for maintaining the electrical excitability of the heart. The direct and indirect effects of cocaine work in tandem to disrupt the co-ordinated electrical activity of the heart and have been associated with life-threatening cardiac arrhythmias. This review focuses on the direct effects of cocaine on cardiac ion channels, with particular focus on sodium, potassium and calcium channels, and on the contributions of these channels to cocaine-induced arrhythmias. Companion articles in this edition of the journal examine the epidemiology of cocaine use (Wood & Dargan [1]) and the treatment of cocaine-associated arrhythmias (Hoffmann [2]).

Introduction

Recreational use of cocaine causes increases in heart rate and blood pressure, effects that can be attributed primarily to its activity on autonomic nerves where cocaine acts as a competitive antagonist of norepinephrine uptake into nerve terminals and increases the systemic concentration of circulating catecholamines [3–5]. β-adrenergic stimulation of the heart is known to modulate calcium metabolism, the activities of sarcolemmal ion channels and myocardial contractility [4, 6]. Circulating catecholamines also act as vasoconstrictors of coronary vasculature leading to ischaemia and myocardial infarction, suspected causes of death in many cases of cocaine abuse [5, 7]. Recent evidence suggests that inherited channelopathies that predispose individuals to lethal arrhythmias may potentiate the cardiotoxic effects of cocaine [8–10].

Although cocaine is known to potentiate arrhythmias caused by pre-existing myocardial diseases [11, 12], several studies point to a more direct pro-arrhythmic role for cocaine. Autopsies of patients who died following cocaine use indicate that in many cases death occurred in healthy individuals who had no evidence of myocardial damage or coronary vascular disease [13–16]. In these patients cocaine may induce arrhythmias by mechanisms that are independent of other predisposing factors. In addition to its effects on heart rate and blood pressure, cocaine also disrupts the co-ordinated electrical activity of the heart causing increases in the PR, QRS and QT intervals of the electrocardiogram (ECG) [17–22]. These electrophysiological changes are generally attributed to the direct effects of cocaine on cardiac ion channels where multiple sites of action are known [13, 15]. Cocaine inhibits L-type calcium (Ca) currents, delayed rectifier potassium (K) currents, and sodium (Na) currents of cardiomyocytes [23–25]. The combination of enhanced sympathetic stimulation, disturbances in cardiac electrophysiology, pre-existing heart disease and genetic variants that increase susceptibility to

arrhythmias may act in concert to produce the potent cardiotoxic effects of cocaine in humans. This review focuses on evidence for direct modulation of voltage-dependent cardiac ion channels (specifically, Na, K and Ca channels) by cocaine and the roles such direct effects play in cocaine-induced arrhythmia.

Cocaine slows the conduction of myocardial electrical impulses

One of the hallmarks of cocaine toxicity is a reduction in myocardial conduction velocity, a well-known risk factor for cardiac arrhythmias [13, 14, 19, 20, 24]. Voltage-gated Na channels play a key role in the electrical excitability of the myocardium and are responsible for the rapid upstroke of the cardiac action potential (AP). Within the range of concentrations known to cause acute toxicity in humans (1–70 μM) [12], cocaine produces a potent inhibition of cardiac Na currents [24, 26] and prolongs the refractory period for the generation of APs [27]. Cocaine-induced inhibition of Na channels and slowing of myocardial conduction underlie the prolonged QRS interval associated with the use of this drug [24].

Alcohol is frequently consumed by individuals who use cocaine [28, 29] because it is perceived to potentiate and prolong the effects of the drug [30, 31]. The combination of ethanol and cocaine has been shown to increase heart rate and blood pressure beyond what is observed for the individual drugs and increases the risk of adverse cardiac events [30, 32–34]. Cocaethylene, a metabolite of cocaine and ethanol [35], slows cardiac conduction, delays repolarization [36] and is a potent inhibitor of both Nav1.5 sodium and human ether-à-go-go-related gene encoded potassium channels [37–40]. Cocaethylene inhibition of cardiac ion channels contributes to the increased incidence of cardiac arrhythmias associated with the combined use of cocaine and alcohol [41–43].

Cocaine inhibition of cardiac sodium currents

Cellular electrophysiological recording from dissociated cardiomyocytes and heterologous expression systems has been widely used to investigate the effects of cocaine on voltage-gated Na channels [24, 26, 44–47]. Within a clinically relevant range of concentrations (<30 μM) cocaine produces little inhibition of Na currents when cardiomyocytes are held at a hyperpolarized potential and stimulated at low frequency [24]. This observation is consistent with the proposal that cocaine does not bind appreciably to Na channels under resting conditions. The effects of cocaine have been further investigated using the heterologously expressed Nav1.5 channel. Nav1.5, encoded by the *SCN5A* gene, is the predominant Na channel expressed in cardiac tissues and is an important determinant of electrical excitability. Increasing the stimulation frequency produces a progressive reduction in Nav1.5 current amplitude for successive pulses within a stimulation train by a mechanism commonly referred to as use-dependent inhibition (Figure 1A). The finding that rapid repetitive depolarization enhances cocaine inhibition indicates that Na channel gating (i.e. activation, inactivation) plays an important role in cocaine binding. An essential component of the cocaine-induced use-dependent inhibition is that the drug-modified channels do not fully recover during the short rest interval between depolarizing pulses. In the absence of cocaine the majority of cardiac Na channels rapidly recover from inactivation at hyperpolarized voltages with time constants (τ) of the order of 10 ms (Figure 1B). Cocaine substantially reduces the fraction of Na channels recovering with the rapid time constant and dramatically slows the recovery of drug-modified channels ($\tau = 7.5$ s). Drug binding prevents Na channels from fully recovering between stimulation pulses resulting in use-dependent inhibition. Cocaine also produces an apparent shift in steady-state inactivation that selectively reduces the availability of Na channels at voltages near the resting membrane potential. By binding preferentially to inactivated states, cocaine alters the steady-state equilibrium resulting in channels that appear to inactivate at more hyperpolarized voltages (Figure 1C). As with use-dependent inhibition, the slow recovery of cocaine-modified channels underlies this shift in availability. These data support a model in which cocaine binds preferentially to open and inactivated states and stabilizes Na channels in a non-conducting inactivated state from which they slowly recover at hyperpolarized voltages.

Cocaine binding to sodium channels is state-dependent

The observed effects of cocaine are consistent with the basic tenants of the modulated receptor hypothesis (MRH), a widely employed model used to describe the binding of local anaesthetics [48, 49]. MRH is rooted in the principle that the affinity of local anaesthetic binding varies with the gating state of the channel with the open (O) and inactivated (I) states having higher affinity than closed (C) states (Figure 2). The model proposes that under resting conditions, the interaction of anaesthetics with closed Na channels (C→CD) is minimal because the binding site is in its low affinity conformation and access to the site is barred by the closed activation gate. Depolarization has two effects: i) the channels open permitting drugs to access rapidly the binding site through the cytoplasmic aqueous pathway (O→OD) and ii) it converts the anaesthetic binding site to its high-affinity conformation. After several milliseconds of depolarization the channels begin to inactivate (O→I), which is known to stabilize further the

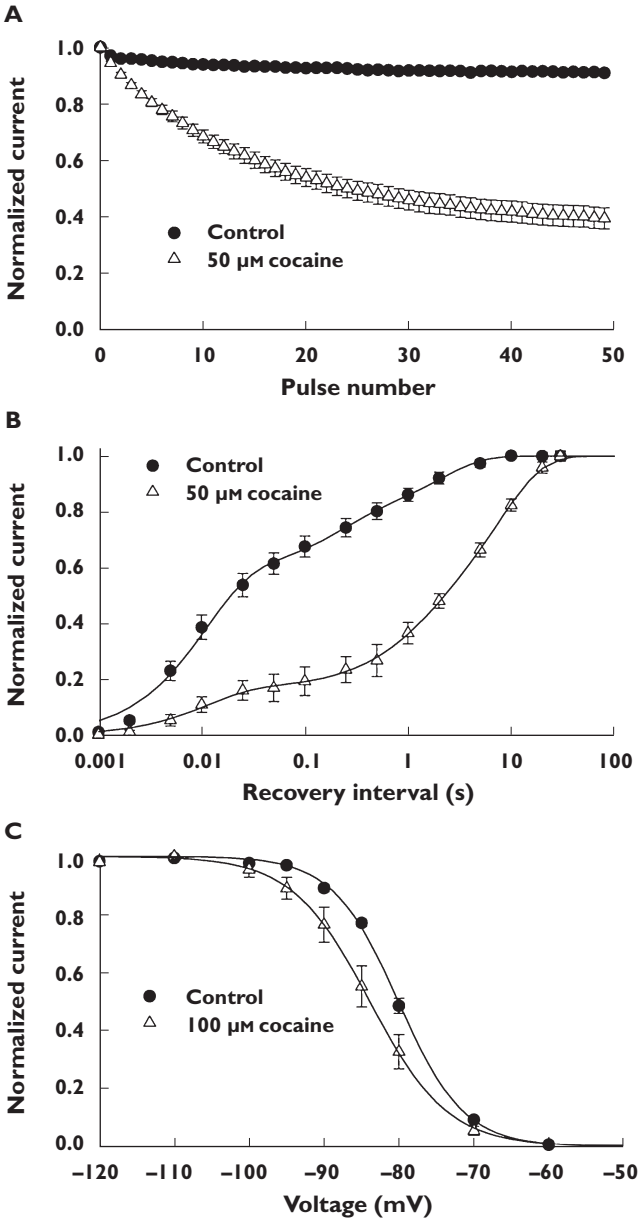

Figure 1

Properties of cocaine inhibition of cardiac Na channels. Effects of cocaine on cardiac (Nav1.5) Na channels heterologously expressed on *Xenopus* oocytes were investigated. (A) Use-dependent inhibition induced by applying depolarizing pulses at a frequency of 5 Hz. (B) Recovery from inactivation measured in the absence and presence of cocaine. In control experiments the channels recovered with time constants of 11 and 189 ms. After application of cocaine the majority of the channels (77%) recovered with a time constant of 7.5 s. (C) Steady-state inactivation was measured by applying depolarizing prepulses between −120 and −60 mV for 60s. The smooth curves are fits to a Boltzmann function with mid-points of −80 mV for control and −84 mV after application of cocaine. Figure reproduced with permission from O'Leary [47]

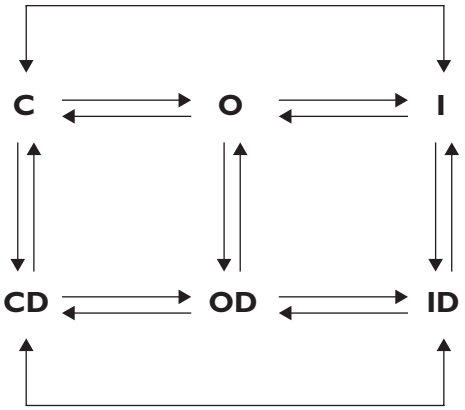

Figure 2

State-dependent drug binding to Na channels. Modulated receptor model describing the state-dependent binding of local anaesthetics. C, O and I represent the closed, open and inactivated states of Na channels, respectively. CD, OD and ID are the drug-modified (D) equivalents. Anaesthetics generally bind with low affinity to closed channels (C) and high affinity to the open (O) and inactivated (I) states. Drugs rapidly access the binding site on open channels (O→OD) through the aqueous pore. Access to the binding site on the closed (C→CD) and inactivated (I→ID) states is through an intrinsically slower hydrophobic pathway

binding of many local anaesthetics (I→ID). Consequently, prolonged depolarization beyond what is necessary to rapidly inactivate Na channels (1–5 ms) frequently promotes high-affinity binding. The time course of anaesthetic binding to inactivated channels (I→ID) is generally slower than what is observed for open channels (O→OD) but in most cases has similar, if not higher binding affinity [48–50]. Unlike the block of open channels where drugs gain access to the binding site through the permeation pore, the inactivation gate of Na channels is believed to act by plugging the cytoplasmic entrance of the channel thus preventing drug access to the receptor via the aqueous pathway [51]. To account for anaesthetic binding to inactivated states a second hydrophobic pathway that permits uncharged forms of these drugs to access the binding site has been proposed [48, 49]. Small lipophilic and neutral anaesthetics gain access to the binding site on inactivated Na channels more readily than the large water-soluble drugs suggesting that size, hydrophobicity and charge play important roles in drug binding via this pathway [52].

A prominent feature of modulated receptor models is that the affinity of drug binding is linked to the gating state of the channel. Consequently, manipulations that interfere with the transition of Na channels into high-affinity open and inactivated states are predicted to weaken drug binding. The link between Na channel gating and drug binding has been explored by exposing Na channels to enzymes, modifying reagents and toxins that disrupt fast inactivation [53–56]. Although the data generally support the concept that inactivation contributes to local anaes-

thetic binding, the findings are complicated by the non-specific effects of these treatments, which in addition to disrupting fast inactivation may alter other properties of the channel [57–59]. More recent studies employ mutations of the interdomain D3-D4 linker of Na channels, the putative inactivation gate, that selectively eliminate fast inactivation [60–63]. This more focused approach offers distinct advantages in that the mutation sites are well defined and physically separated from those that directly participate in local anaesthetic binding [64, 65]. D3-D4 linker mutations that remove fast inactivation substantially reduce the cocaine-induced use-dependent inhibition and accelerate the recovery of the drug-modified channels [47]. These studies indicate that preventing cardiac Na channels from adopting the high-affinity inactivated conformation weakens cocaine binding. This conclusion is further supported by steady-state measurements showing that the affinity of cocaine binding increases >20-fold as Na channels shift between the closed (K_D = 235 μM) and inactivated (K_D = 10 μM) states [24, 66, 67].

A rapid component of cocaine inhibition is observed in native cardiac Na channels that display properties consistent with a pore-blocking mechanism [24]. This component of cocaine inhibition has been further investigated by using D3-D4 linker mutations that eliminate fast inactivation [47]. Cocaine produces a time- and concentration-dependent inhibition of the non-inactivating Na current (Figure 3A). The dissociation constant (K_D) of cocaine binding to the open channels (K_D = 122 μM) is greater than that of inactivated channels (K_D = 4 μM) suggesting that at clinically relevant concentrations (<50 μM) the cocaine block may not significantly contribute to the observed Na channel inhibition. However, unlike the inactivation-dependent component, the cocaine block of open channels displays strong voltage dependence at depolarized voltages suggesting that the drug traverses approximately 50% of the membrane electric field from the internal side to reach its binding site [68]. Similar voltage sensitivity has been observed for local anaesthetics and other blockers and is consistent with a binding site situated deep within the cytoplasmic aqueous pore [54, 63, 69, 70]. Electrostatic interaction with the membrane electric field promotes binding by driving the positively charged drugs onto their binding sites. An important consequence of this voltage-dependence is that at the peak of the cardiac AP (+40 mV) the affinity of cocaine block of open channels (K_D = 14 μM) approaches what is observed for inactivated (K_D = 4 μM) states [47]. Overall, the data are consistent with a model in which cocaine binding to both the open and inactivated states contributes to Na channel inhibition during the cardiac AP.

The evidence is consistent with a model in which the cocaine binds to a site located near the cytoplasmic entrance of Na channels. Channel opening facilitates cocaine binding by creating an aqueous pathway that enables the drug to access rapidly its binding site. Because depolarization causes cardiac Na channels to open briefly (<1 ms) before inactivating [71], only a small fraction of the open Na channels bind cocaine during a cardiac AP. However, cocaine binding to open channels is further stabilized as the Na channels inactivate. The rapid cocaine block of open channels coupled with more stable binding to inactivated channels act in a cooperative fashion to produce the observed use-dependent inhibition of cardiac Na channels (Figure 1A).

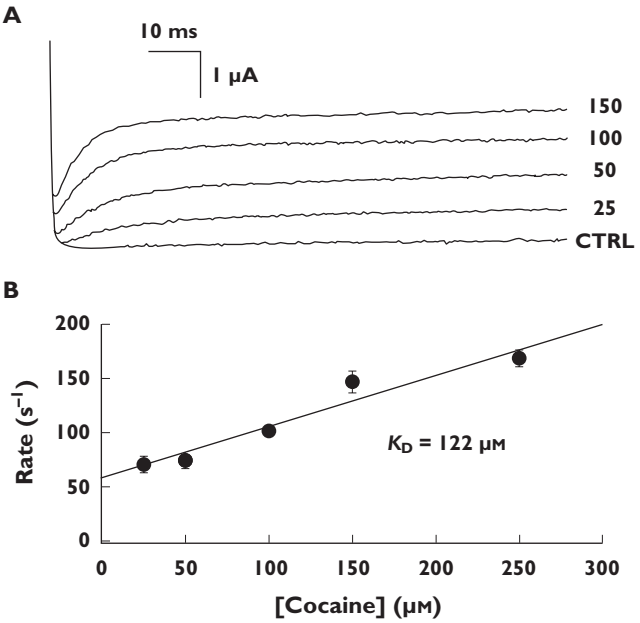

Figure 3

Cocaine block of an inactivation-deficient Na channel mutant. The inactivation-deficient mutant was constructed by replacing hydrophobic residues of the interdomain D3-D4 linker of the cardiac (Nav1.5) Na channel with glutamines (IFM→QQQ) [64]. The mutant channels were heterogously expressed in *Xenopus* oocytes and Na currents recorded using two-electrode voltage clamp. (A) Currents of non-inactivating mutant before (CTRL) and after application of cocaine (25–150 μM). (B) The decay of the current was fitted with an exponential function and the apparent blocking rate (1/τ) plotted *vs.* the cocaine concentration. The straight line predicts a K_D for cocaine binding at −10 mV of 122 μM. Figure reprinted with permission from O'Leary & Chahine [47]

Molecular studies of the cocaine binding site of Na channels

The S6 membrane-spanning segments of all voltage-gated Na channels include highly conserved amino acids that line the cytoplasmic entrance of the channels and contribute to the binding of local anaesthetics [65, 67, 72–75]. Mutagenesis of the neuronal (Nav1.2) Na channel identified two aromatic residues (phenylalanine, tyrosine) of the S6 segment of homologous domain four (D4S6) that appear to be exposed within the cytoplasmic aqueous

pore where they directly contribute to local anaesthetic binding [65]. These residues are situated near the middle (F1760) and C-terminus (Y1767) of the D4S6 and are separated by approximately 11 Å, a distance that is appropriate for the binding of most clinically useful anaesthetics ranging between 10 and 15 Å in length [76]. Substituting non-aromatic residues at these positions in the cardiac (Nav1.5) Na channel reduces cocaine-induced use-dependent inhibition and accelerates the recovery of the drug-modified channels [47, 67]. Mutations of the tyrosine produce a more marked reduction in use-dependent inhibition suggesting that this residue may play a more prominent role in cocaine binding [47]. This differs from many conventional local anaesthetics, where the phenylalanine has generally been shown to play a more critical role in binding [72]. Differences in the chemical structure or physical properties of cocaine and local anaesthetics appear to cause these drugs to adopt slightly different orientations within the D4S6 binding site. The membrane-spanning D1S6 segment of the skeletal muscle (Nav1.4) Na channel has also been implicated in cocaine binding suggesting that residues of the D1S6 may form a common interface with the D4S6 segment [77]. Cocaine and local anaesthetics share an overlapping binding site located near the cytoplasmic entrance of Na channels, which may explain their similar mechanisms of action. Interestingly, the class Ib anti-arrhythmic drug lidocaine displays rapid binding kinetics by comparison with cocaine and appears to displace cocaine competitively from its binding site on native cardiac Na channels [78–80]. Significantly, lidocaine is being investigated as a potential antidote for cocaine-induced arrhythmias [81, 82].

Consequences of cocaine binding within the aqueous pore of Na channels

The binding of cocaine within the cytoplasmic aqueous pore of Na channels has important implications. Studies have demonstrated cocaine inhibition of Na current at both the whole-cell and single-channel levels [68, 83]. The data are consistent with an open-channel blocking mechanism where cocaine binding within the pore prevents Na^+ ions from permeating through the channel. Additional support for this mechanism is obtained from studies showing that raising the external concentration of Na^+ ions weakens the cocaine inhibition and reduces the affinity of cocaine binding [47, 68]. This effect is specific for changes in external Na^+ concentration as equivalent changes in internal Na^+ concentration have little effect on cocaine binding [68]. External Na^+ and internal cocaine appear to occupy distinct but overlapping binding sites within the Na channel pore. Electrostatic interaction between the positively charged cocaine and Na^+ appears to prevent both sites from being simultaneously occupied. Antagonism of cocaine binding by external Na^+ may contribute to the beneficial effects of hypertonic sodium solutions on cocaine-induced electrical disturbances [11, 17, 84].

Clinically useful local anaesthetics have pKa values between 7.7 and 9.5 causing these drugs to be in rapid equilibrium between positively charged (protonated) and neutral (unprotonated) forms at physiological pH [85]. The neutral forms of anaesthetics are able to permeate biological membranes and when applied externally these drugs readily gain access to the cell cytoplasm. This is consistent with data showing that the potency of anaesthetics is generally higher when applied in alkaline solutions that favour the uncharged (i.e. membrane-permeant) forms of the drugs [83, 86]. The positively charged forms of anaesthetics are less membrane-permeant but are generally more potent Na channel inhibitors when applied from the cytoplasmic side [50]. Anaesthetics permeate cell membranes in their neutral forms before converting to their positively charged forms within the cytoplasmic compartment [87, 88].

Cocaine has a pKa of 8.7 so that at physiological pH (7.2) the drug is predominately (97%) charged. Only neutral cocaine, totalling 3% of the externally applied drug concentration, is capable of gaining access to membrane-bound Na channels and accounts for the bulk of Na channel inhibition. This is consistent with data showing that elevating the external pH increases the fraction of neutral cocaine and enhances the inhibition of cardiac Na channels [83]. In addition to determining the partitioning of cocaine between the aqueous and lipid phases, external pH also has a direct effect on cocaine binding. Reducing external pH has been shown to stabilize cocaine binding and slow the recovery of drug-modified channels [68, 83, 89]. These findings are consistent with studies showing that local anaesthetic binding to Na channels is stabilized by reducing extracellular pH while equivalent changes in the internal pH have little effect on drug binding [48, 90–94]. These findings indicate that external protons permeate through the channel pore where they interact with intracellular anaesthetic converting the bound drugs to their more potent charged forms. Changes in external pH have little effect on the binding of permanently charged quaternary derivatives of local anaesthetics indicating that extracellular protons produce changes in binding by acting on the drug rather than the channel protein [89].

Seizures are common events during severe cases of cocaine overdose [95] and are often associated with substantial reductions in the arterial pH [11, 96]. Enhanced cocaine binding at low pH may exacerbate Na channel inhibition and further slow electrical conduction within the myocardium. This is supported by data indicating a close relationship between arterial pH and QRS intervals in cocaine-intoxicated patients [97]. Systemic administration of hypertonic sodium bicarbonate solutions that restore physiological pH produce a rapid reversal of the cocaine-induced widening of the QRS interval by relieving Na

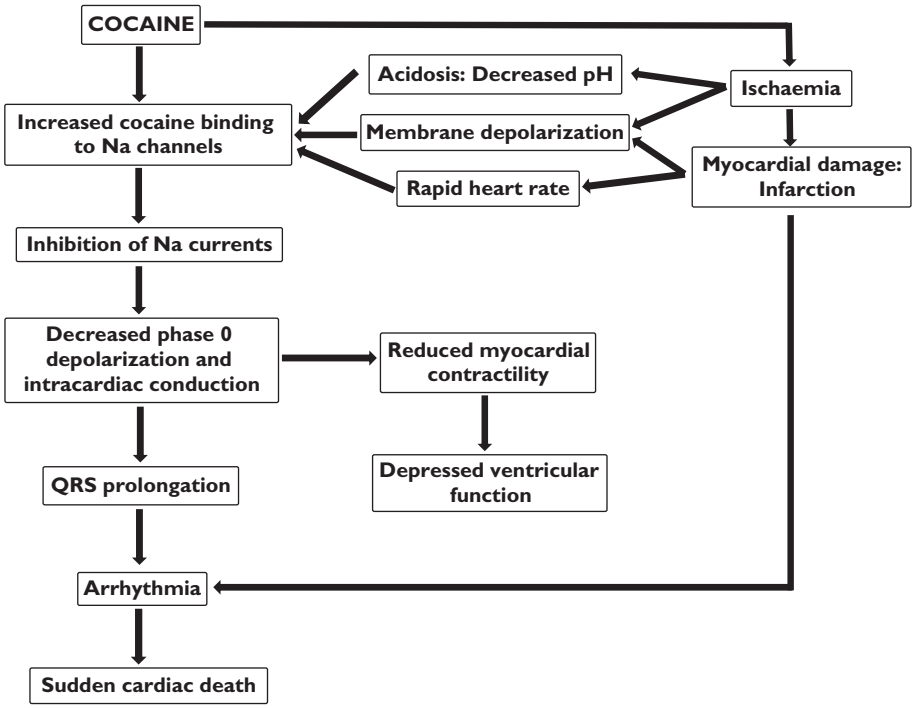

Figure 4

Schematic of cocaine-induced cardiotoxicity related to Na channel inhibition. Cocaine stabilizes Na channels in inactivated states that do not conduct Na current. Na channel inhibition slows the rapid upstroke of the AP (Phase 0 depolarization), an important determinant of intracardiac conduction. Slowed conduction decreases myocardial contractility leading to depressed left ventricular function and haemodynamic impairment. Prolongation of ventricular depolarization (QRS interval) exposes the myocardium to potentially lethal re-entrant arrhythmias. Combining Na channel inhibition with other pro-arrhythmic electrical disturbances, such as the inhibition of delayed rectifier hERG (long QT intervals) or L-type calcium channels, further increases the likelihood of arrhythmias and sudden cardiac death. Cocaine-induced ischaemia caused by enhanced sympathomimetic stimulation of coronary vasculature can lead to myocardial damage that further potentiates Na channel inhibition and cardiac arrhythmias. Cocaine binding is enhanced at rapid heart rates due to use-dependent inhibition, under conditions where the resting membrane potential is depolarized due to high-affinity binding to inactivated states and during episodes of acidosis, which stabilizes cocaine in its more potent positively charged form

channel inhibition [17, 79]. The combination of elevated pH and increased extracellular Na^+ concentration appears to work in concert to weaken cocaine binding to Na channels and reduce the pro-arrhythmic potential of this drug. Figure 4 summarizes the important determinants of cocaine binding to cardiac Na channels and illustrates the synergistic relationship between the direct and indirect cardiotoxic effects of cocaine.

Cocaine and torsades de pointes

Although monomorphic ventricular tachycardia associated with inhibition of I_{Na} may be the most common arrhythmogenic effect of cocaine, it is not the only one. There is evidence in the literature that cocaine can also induce torsades de pointes (TdP), a polymorphic ventricular tachycardia characterized by twisting of the QRS axis around the isoelectric line of the ECG [15, 98]. Drug induced TdP is typically associated with QT interval prolongation [99, 100] and it is notable that cocaine-induced TdP has been reported in individuals with idiopathic long QT syndrome [101, 102] and in patients taking concomitant medication that confers an independent risk of QT interval prolongation and TdP, particularly methadone or levomethadyl [103–105]. A study of the short-term, acute effects of smoking cocaine on habitual cocaine users showed significant prolongation of rate-corrected QT (QT_C) interval, smaller T wave amplitude and more marked U wave [106]. Electrocardiogram analysis of patients hospitalized for cocaine abuse has shown marked evidence of altered repolarisation, including QT_C interval prolongation, and increased QT_C dispersion [107, 108]. In one analysis of emergency room patients treated for cocaine abuse, 26% of ECGs exhibited QT_C intervals > 440 ms, whilst a higher figure of 75% was found amongst a small number of subjects who died suddenly [107]. In another study, patients were divided into two groups on the basis of presence or absence of anginal chest pain, with both QT_C prolongation and dispersion being more prominent in the chest pain group; this may suggest an increased risk when abnormal repolarization is combined with myocardial ischaemia

[108]. The risk of TdP may also be exacerbated when cocaine is taken in combination with alcohol [109].

The mechanisms by which drugs lead to QT interval prolongation and TdP have been investigated intensively. Excessively delayed ventricular repolarization is associated at the cellular level with early-afterdepolarizations (EADs), depolarizing events that are superimposed on AP repolarization and that are implicated as potential trigger events for TdP arrhythmia; at the tissue level increased dispersion of repolarization may form a substrate for re-entrant tachycardia (see [99, 100] for reviews). The majority of torsadogenic agents share a common cellular mechanism of action, which is to inhibit a cardiac K current called the 'rapid delayed rectifier (I_{Kr}) [99, 100, 110, 111]. Accordingly, the role of I_{Kr} in ventricular repolarization and TdP will briefly be considered, followed by evidence that the channels mediating I_{Kr} are targets for pharmacological inhibition by cocaine and its metabolites.

I_{Kr} and ventricular repolarization

I_{Kr} was identified as a pharmacologically and biophysically distinct repolarizing K current through the use of an experimental Class III anti-arrhythmic agent, E-4031 [112]. The pore-forming sub-unit of the channel was later identified as being encoded by *human ether-à-go-go related* gene (*hERG* alternative nomenclature *KCNH2*), mutations to which were associated with one form (LQT2) of congenital long QT syndrome [113–116]. Both native I_{Kr} and heterologously expressed hERG channels display unique biophysical properties (for reviews see [100, 111, 116, 117]). Figure 5 illustrates schematically the role of I_{Kr} channels in ventricular repolarization. Due to a rapid voltage-dependent channel 'inactivation' process, early during the ventricular AP, these channels make relatively little contribution to AP repolarization, but they make a greater contribution as the AP plateau declines and inactivation is relieved, with I_{Kr}/hERG current being maximal late in repolarization, prior to the terminal repolarization phase of the AP [117–121], during which a different potassium current (the inwardly rectifying K current, I_{K1}) predominates [111, 122, 123]. Consequently, I_{Kr} is well suited to regulate ventricular AP duration and, thereby, the QT interval of the ECG. Due to the fact that I_{Kr} is so important for regulating AP repolarization, drugs that impair I_{Kr} function produce marked effects on AP duration and, consequently, upon the QT interval duration of the electrocardiogram [99, 100, 110, 111].

Cocaine prolongs ventricular action potential duration and inhibits I_{Kr}

A direct effect of cocaine on ventricular repolarization at the cellular level was demonstrated by Kimura and colleagues [25]. They observed feline ventricular myocyte AP prolongation with 10 and 50 μM cocaine [25]. At the higher concentration, cocaine also elicited EADs. Cocaine-induced EADs were exacerbated by isoprenaline and were suppressed by the L-type calcium channel inhibitor verapamil [25]. A key role has been identified for L-type calcium channels during delayed ventricular repolarization, in mediating Ca^{2+} ion entry that gives rise to EADs [100, 110, 124, 125]; this is concordant with the effect of verapamil on cocaine-induced EADs in this study [25]. Net outward delayed rectifier current was also inhibited by cocaine, whilst the inward rectifier current I_{K1} was unaffected. Clarkson and colleagues [23] subsequently demonstrated marked (>20%) prolongation of guinea-pig ventricular APs by 3 μM cocaine, whilst high cocaine concentrations led to AP shortening. In this study both I_{K1} and the 'slow' component of delayed rectifier K current (I_{Ks}) were comparatively little affected by low concentrations of cocaine, whereas I_{Kr} was markedly affected with a half-maximal inhibitory concentration (IC_{50}) of ~4 μM [23]. The overlap between AP-prolonging and I_{Kr}-blocking concentrations of cocaine in that study [23] is consistent with the notion that I_{Kr} block mediates the AP prolonging effect of cocaine. A subsequent study has demonstrated that cocaine can also inhibit ionic current (I_{KATP}) carried by cardiac ATP-sensitive K (K_{ATP}) channels (IC_{50} 9 μM) [126]. These channels are inhibited by normal intracellular levels of ATP and become activated during ischaemia; this gives rise to AP shortening

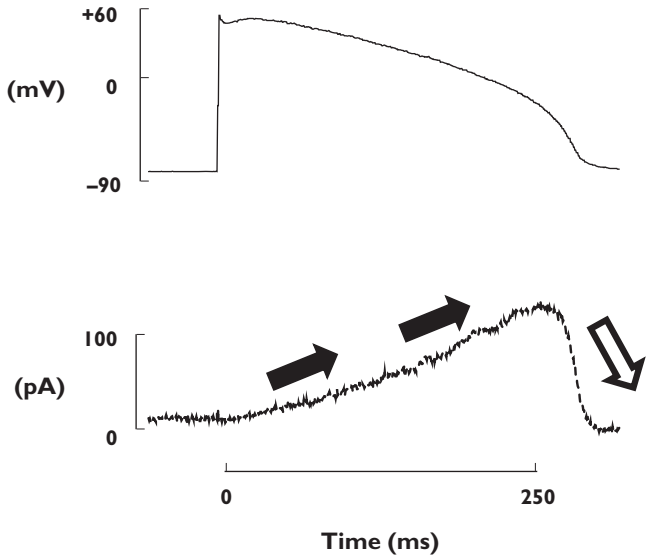

Figure 5

Role of I_{Kr} in ventricular action potential repolarization. Schematic diagram showing the profile of I_{Kr} (lower trace) during the ventricular action potential (upper trace). Outward current flow via I_{Kr} increases throughout the plateau phase of the action potential (marked by filled arrows), peaking before terminal repolarization, during which I_{Kr} declines (marked by open arrow)

which may protect myocytes from calcium-overload [123]. Due to the fact that K_{ATP} channels are generally activated under conditions of metabolic stress [123], K_{ATP} channel block seems unlikely to mediate AP prolonging effects in the well-perfused myocardium. However, it has been suggested that this effect may be relevant in the context of acute ischaemic preconditioning, which may ensue from coronary spasm with cocaine exposure [126].

hERG and cocaine: the molecular basis of I_{Kr} channel inhibition

Studies of recombinant hERG channels expressed in mammalian cell lines have advantages over native I_{Kr} in that i) heterologous expression systems lack the overlapping currents in cardiomyocytes that can mask drug effects on I_{Kr} and ii) the amplitude of ionic currents measured through overexpressed hERG channels is greater than that through native I_{Kr} [100, 127]. These features facilitate mechanistic investigation of channel blockade. Effects of cocaine on I_{hERG} have been assessed using hERG channels expressed in tsA201 cells, with marked I_{hERG} inhibition observed at micromolar concentrations during both conventional voltage-clamp experiments and during experiments performed using the action potential voltage clamp (AP clamp) technique [128]. The observed IC_{50} for cocaine inhibition of I_{hERG} elicited by voltage step protocols was 5.6 μM (Figure 6A), whilst marked inhibition was also observed under AP clamp (Figure 6B,C) [128]. Further experiments in the same study pursued the mechanism of the inhibitory effect, the results of which suggested that cocaine binds preferentially to gated hERG channels in activated or inactivated channel states [128]. An independent study published the same year, using HEK293 cells as expression system, reported a similar IC_{50} for I_{hERG} inhibition of 7.2 μM, whilst demonstrating that high concentrations of cocaine were unable to inhibit channels encoded by KCNQ1+KCNE1 (the genes responsible for α and β subunits that recapitulate native I_{Ks}; old nomenclature KvLQT1 + minK) [129], consistent with the earlier observation of comparative insensitivity to cocaine of ventricular I_{Ks} [23]. Application to the cell interior via the patch pipette solution of N-methyl-cocaine, a charged membrane impermeant cocaine analogue, demonstrated that cocaine most likely acted through binding to a site accessible from the interior of the cell membrane [129]. A third study (also using the HEK293 cell expression system) reported an IC_{50} for I_{hERG} inhibition of 4.4 μM [39], providing further independent verification of significant inhibitory activity in the low micromolar concentration range.

Additional insight into the hERG-blocking properties of cocaine has been obtained through the study of the I_{hERG}-blocking propensity of cocaethylene, an ethyl homologue of cocaine that would be anticipated to be formed *in vivo* as a product of cocaine and alcohol co-ingestion [39, 40].

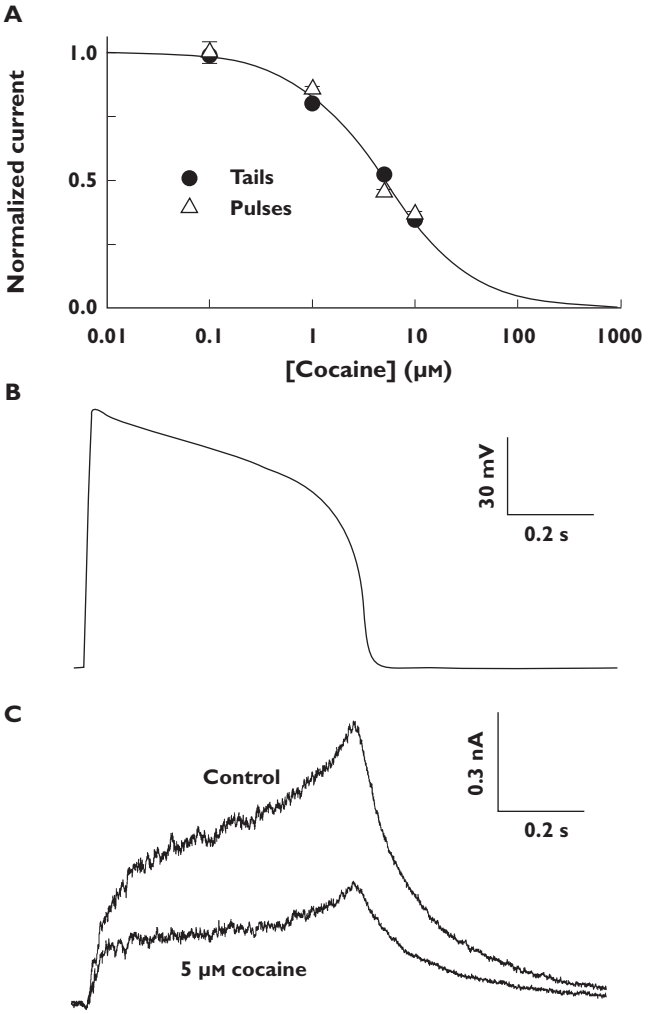

Figure 6

Inhibition of I_{hERG} by cocaine. (A) shows concentration–response relations for inhibition by cocaine of I_{hERG} recorded from hERG-expressing tsA201 cells. I_{hERG} was elicited by depolarization to +20 mV and tail currents were observed on repolarization to −80 mV. Concentration–response relations were similar whether I_{hERG} amplitude was measured for currents during the depolarizing pulse or for current tails. The calculated IC_{50} value was 5.6 ± 0.4 μM. (B) shows ventricular action potential waveform used for AP clamp experiments in which effects of cocaine on I_{hERG} from ts201 cells were studied. (C) I_{hERG} in standard external solution (control) and following exposure to 5 μM cocaine. Data are reproduced from O'Leary [128] with permission

In two independent studies, cocaethylene was found to inhibit I_{hERG} with IC_{50} values of 1.2 μM [39] and 4.0 μM [40] exhibiting properties of a predominantly open channel blocker [39, 40]. By contrast, other metabolites of cocaine have been reported to be markedly less potent I_{hERG} inhibitors [39]. Thus, cocaine's major pyrolysis metabolite methylecgonidine inhibited I_{hERG} with an IC_{50} of ~170 μM, whilst ecgonine methylester or benzoylecgonine produced only modest levels of I_{hERG} inhibition (~20% or less) at 1 mM [39]. Collectively, these observations indicate that cocaine inhi-

bition of native I_{Kr} is mediated by drug blockade of the pore-forming hERG subunit and that both the parent compound and, in the setting of concurrent alcohol intoxication, cocaethylene are likely to produce hERG/I_{Kr} inhibition.

The remarkable sensitivity of hERG channels to pharmacological inhibition by diverse drugs is related to particular structural features of the channel [100, 117, 123, 130]. These include a relatively large pore cavity and the presence of aromatic amino acids (a tyrosine at position 652 (Y652) and a phenylalanine at position 656 (F656)) in the inner (S6) helices of the channel; for some drugs conformational changes to the channel due to rapid voltage-dependent inactivation are also important to stabilize drug binding [100, 117, 123, 130–132]. The molecular basis of cocaine inhibition of hERG has been investigated using wild-type (WT) and mutant hERG channels expressed in HEK293 cells [133]. Inhibition of WT I_{hERG} in this study occurred with IC_{50} values between ~9 and ~14 μM depending on external potassium concentration, though these differences were not statistically significant [133]. The use of a range of inactivation-modifying mutations showed that hERG inhibition by cocaine does not depend on the process of hERG channel inactivation, although mutation of one residue (S620) could impair drug binding independent of any role in the inactivation process. Mutation of nearby inner helical residues (T623, S624) impacted significantly on potency of inhibition (with IC_{50} values for T623A and S624A mutations of ~167 and ~45 μM), whereas alanine mutation of the aromatic residue Y652 increased the IC_{50} to ~310 μM. Mutations of the nearby aromatic residue F656 also impacted significantly on blocking potency, with a strong correlation between hydrophobicity of the residue at position F656 and observed blocking potency [133]. Residues shown to impact significantly on cocaine binding to hERG are indicated schematically in Figure 7.

To summarize, cocaine inhibits hERG channels by binding within the channel pore cavity, gaining access to its binding site on channel gating. Once in the channel it interacts with aromatic S6 residues, with the observed level of blockade also influenced by inner helical residues.

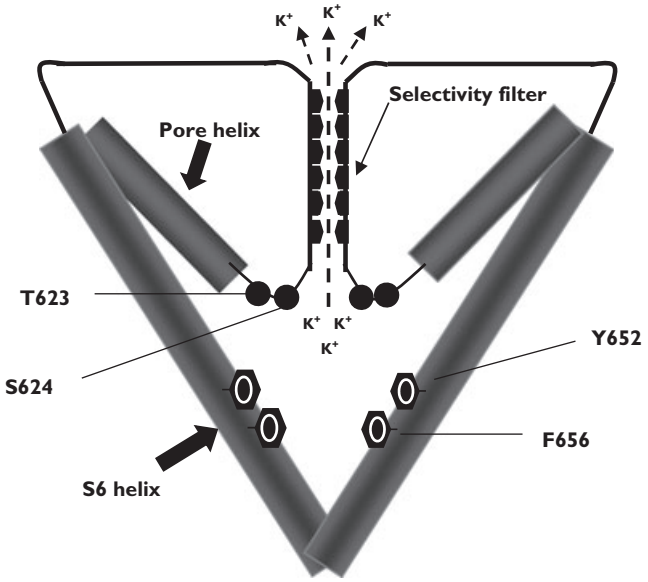

Figure 7

Schematic diagram showing amino-acid residues on hERG implicated in cocaine binding. The figure shows a vertical cross section through two of the four subunits that comprise functional hERG channels, focusing on the S6 and pore helical regions implicated in drug binding. The selectivity filter of the channel and direction of outward flux (as would occur physiologically through open channels) of K^+ ions are also shown. Aromatic residues Y652 and F656 are implicated in the binding of a range of drugs and their mutation influences cocaine binding [133]. T623 is also critical for cocaine binding, whilst mutation of S624 also influences observed potency of inhibition, though to a less marked extent [133]. Mutation of the nearby S620 (not shown) to threonine, but not cysteine has also been shown to influence potency of cocaine inhibition of I_{hERG} [133]

Effects of cocaine on L-type calcium channels

Ca^{2+} influx into cardiomyocytes through ionic current ($I_{Ca,L}$) carried by L-type calcium channels is essential for initiation of the process of excitation-contraction coupling and also contributes to the plateau phase of ventricular APs [134–136]. Due to contribution of $I_{Ca,L}$ to the AP plateau and its role in mediating Ca^{2+} entry during EADs, agents that modulate $I_{Ca,L}$ can either exacerbate or offset the effects of reduced I_{Kr}/hERG function [100].

By contrast with the clear-cut situation regarding I_{Kr}/I_{hERG}, the effects of cocaine on cardiac $I_{Ca,L}$ are perhaps a little more complex: both inhibitory and stimulatory effects have been reported [23, 25, 137]. In their study of feline ventricular myocytes, Kimura and colleagues observed a modest (23%) inhibitory effect of 50 μM cocaine on peak $I_{Ca,L}$ amplitude, without any concurrent significant alteration of the current's inactivation time course [25]. In their subsequent study of guinea-pig ventricular myocytes, Clarkson and colleagues also observed an inhibitory effect of high concentrations of cocaine (30 and 100 μM being associated with reductions of peak $I_{Ca,L}$ by ~24 and 43%, respectively, in comparison with a 9% reduction in control (cocaine-free) time-matched measurements [23]). These authors also observed a biphasic effect of cocaine on ventricular APs, with lower concentrations prolonging AP duration, whilst high concentrations produced AP shortening [23]. The AP shortening observed at the higher cocaine concentrations studied could be attributable to its inhibitory effect on $I_{Ca,L}$ together with a concomitant inhibitory action on plateau sodium current that was also observed [23].

In contrast with $I_{Ca,L}$ inhibitory effects of high cocaine concentrations, a stimulatory effect of low concentrations

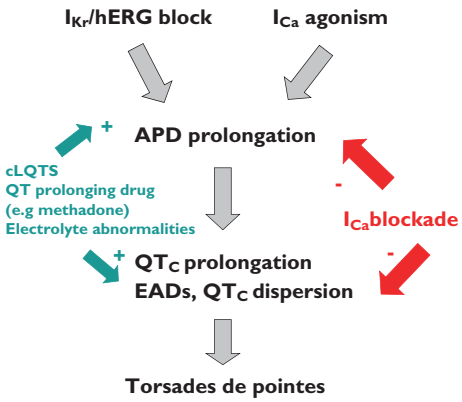

Figure 8

Schematic diagram showing links between hERG, L-type Ca current and cocaine-induced arrhythmia. Vertical 'information flow' (downward arrows) shows consequences of I_{Kr}/I_{hERG} inhibition, namely prolongation of ventricular action potential duration (APD) and consequent QT_c prolongation and QT_c dispersion at the intact tissue/heart level. Delayed repolarization (especially at low rates) predisposes to early afterdepolarisations (EADs). EADs and enhanced dispersion of repolarization (QT_c dispersion) would be anticipated to combine to lead to TdP arrhythmia. At low cocaine concentrations I_{Ca} agonism may exacerbate effects of hERG inhibition. '+' on left hand side of diagram indicate conditions that exacerbate repolarization-delay/TdP risk. '–' on right hand side of diagram indicate where L-type Ca channel inhibition could offset consequences of I_{hERG} inhibition

has been reported [137]. In a study of rat ventricular $I_{Ca,L}$ at both macroscopic and single channel levels, and using both Ca^{2+} and Ba^{2+} ions as charge carriers, Premkumar demonstrated a marked agonist effect of low μM and sub-μM cocaine concentrations, with an observed EC_{50} of 274 nM [137]. The voltage dependence and single channel conductance of $I_{Ca,L}$ were unaffected. However, the channel opening rate was increased whilst closing rate was decreased, which taken together may account for the increase in observed current [137].

An integrated view of the role of I_{Kr}/hERG and Ca channel modulating action of cocaine in arrhythmogenesis

On the basis of clinical and experimental observations in the literature considered in the foregoing sections, it is possible to propose an integrated scheme (Figure 8) by which modulatory effects of cocaine on I_{Kr}/hERG and $I_{Ca,L}$ influence arrhythmia risk.

Cocaine and cocaethylene concentrations up to 784 nM and 1.45 μM in non-fatal trauma victims have been reported [138, 139], whilst average post-mortem bloods concentrations in 'street cocaine' fatalities can attain 20 μM (6.2 mg l^{-1}) [12]. Consequently, at clinically relevant cocaine/cocaethylene concentrations, significant levels of I_{Kr}/hERG blockade can be anticipated to occur, leading to ventricular AP and QT_c interval prolongation, as well as enhanced QT_c dispersion. These effects are likely to be exacerbated (i) when alcohol consumption is combined with cocaine abuse, (ii) when other QT_c interval prolongation agents, particularly methadone, are consumed and (iii) when other risk factors for TdP (e.g. congenital LQTS, electrolyte abnormalities [99, 100]) are present. Ventricular AP prolongation that would result from I_{Kr}/hERG inhibition can be anticipated to increase risk of EADs, and this could be further exacerbated (at least at high nM or low μM cocaine concentrations) by an agonist effect on $I_{Ca,L}$. EADs and increased QT_c dispersion would be anticipated to combine to lead to TdP arrhythmia. Due to the fact that EAD risk is exacerbated at slow rates [100], ventricular pacing might be considered to offset toxic effects of cocaine mediated by delayed repolarization. However, pacing at an increased rate could feasibly exacerbate the I_{Na} inhibitory effect of cocaine. On the other hand, were $I_{Ca,L}$ inhibition to occur, this would be anticipated to offset the cocaine-induced delayed repolarization. It has been noted that K-channel related pro-arrhythmia is likely to occur outside of the emergency room setting, but that for those patients seen in hospital who are considered to be at 'high risk' of TdP, intravenous magnesium may be beneficial [140].

Cocaine inhibition of cardiac ion channels and acute myocardial ischaemia

Although the cardiotoxic effects of cocaine on the heart are well documented, the mechanism by which this drug promotes potentially life-threatening arrhythmias remains controversial. In part, this stems from the pharmacological properties of cocaine, which acts as both a local anaesthetic and a sympathomimetic agent [4, 13, 14]. Enhanced sympathetic stimulation is believe to induce vasoconstriction of coronary arteries that reduces the supply of oxygenated blood to the myocardium thereby promoting infarctions and cardiac arrhythmias [7, 141, 142]. Alternatively, the use of cocaine may be intrinsically pro-arrhythmic capable of inducing lethal arrhythmias in the absence of myocardial damage, coronary vascular disease or ischaemia [12, 143–145]. In addition to its effects on heart rate and blood pressure cocaine prolongs the PR, QRS and QT intervals of the ECG [17–22]. These potentially life-threatening electrical disturbances result from the direct effects of cocaine and its metabolites on Na, K and Ca channels.

The sympathomimetic effects of cocaine and the direct inhibition of cardiac ion channels do not occur in isolation and may be interrelated (Figure 4). A prominent feature of acute myocardial ischaemia is a localized increase in extra-

cellular potassium concentration ranging between 10 and 15 mM within the ischaemic zone [146, 147] resulting in depolarization of the resting membrane potential of cardiomyocytes of 20–25 mV [147, 148, 149]. This K^+-induced depolarization occurs over the range of voltages where cardiac Na channels inactivate (–100 to –60 mV) resulting in an increase in the fraction of inactivated Na channels under resting conditions (Figure 1C). High-affinity cocaine binding to these inactivated channels is predicted to reduce further the availability of the Na channels within the ischaemic area further slowing electrical conduction and increasing the risk of re-entry arrhythmias [150–153]. Another characteristic of ischaemia is acidosis, which can cause the arterial pH to fall by more than a pH unit ($\leq$6.5) [154–157]. Accumulation of H^+ near the external mouth of Na channels may lead to the protonation of internally bound cocaine resulting in more stable drug binding, slowed recovery and further decreases in electrical conduction within the ischaemic zone. Finally, enhanced sympathomimetic stimulation, the principal cause of vasoconstriction, also promotes tachycardia that may exacerbate the cocaine-induced use-dependent inhibition of Na channels (Figure 1A).

Inherited mutations and drugs that reduce hERG currents have been shown to produce long QT syndromes that predispose healthy individuals to ventricular arrhythmias and sudden death [158, 159]. These lethal events occur in the absence of other complications such as pre-existing heart disease [158] or ischaemia [160]. Because of its block of hERG channels, cocaine produces an acquired form of long QT syndrome that may further increase arrhythmogenicity. A suspected trigger for sudden death in patients with long QT syndromes is emotional or physical stress, which is believed to act via an increase in sympathetic stimulation [158]. Recent work suggests this may be mediated by adrenergic regulation of hERG channels [161]. Finally, lowering extracellular pH reduces hERG current amplitude and alters gating kinetics, effects that are expected to prolong further the QT interval [162, 163]. The downstream events associated with acute myocardial ischaemia (i.e. $\downarrow$pH, $\uparrow[K^+]_o$) may enhance cocaine binding to cardiac Na and K channels further exacerbating the electrical disturbances and increase the risk of cardiac arrhythmias (Figure 4).

Competing interests

None declared.

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